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Endovascular Methods for Stem Cell Transplantation

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1. Introduction

Results from cell transplantation research have received interest and attention both from a clinical, a scientific and a public point of view. This chapter discusses new endovascular transplantation methods for different cell systems. First different cell based therapies are presented, followed by an overview of pathological conditions wherein several cell based strategies are implemented. Thereafter the delivery of cells in broad terms, and then specifically by endovascular technique compared to surgical technique, is presented. A general description of the active process of diapedesis is provided, as it is understood for immunological cells, since this is most probably a fundamental process for endovascular transplantation of cells as well.

1.1 Cell based therapies

Cell based strategies are sought after as a way of repairing or facilitating self renewal in pathological organ systems that have little or no intrinsic regenerative capacity. The plethora of different diseases in organ systems that might have a regenerative capacity, but is limited through physiological processes, is almost boundless (Bajada et al. 2008). Cell based therapies have been successfully used in the clinical practice for distinct pathological conditions during a relatively long period of modern medicine. One of the broadest success stories are the transplantation of cells to patients suffering from hematological diseases (Buckner et al. 1974; Thomas et al. 1975; Thomas et al. 1975; Slavin et al. 1998). Hematological stem cell transplantation has also been expanded to comprise autologous transplantations following chemotherapy of solid tumor forms (Childs et al. 2000). Following the isolation of stem cell lines from human blastocysts (Thomson et al. 1998) and other adult sources such as the central nervous system (CNS) (Johansson et al. 1999), bone marrow (Bruder et al. 1997), multi-lineage mesenchymal (Pittenger et al. 1999), adipose tissue (Zuk et al. 2001) among others, new cell based approaches to disease treatment can be envisioned. The potential for *in vivo* expansion of these cells followed by transplantation, re-implantation and/or tissue engineering becomes possible (Vacanti et al. 1999). These findings open up possibilities for strategies aimed at ameliorating disease burden, or in the long run, obtaining curative goals through cell therapies in a clinical setting. Proposed treatments can broadly be divided into stimulation of an endogenous population or

transplantation of cells (Lindvall et al. 2006), be them homologous, from a donor or across the xeno-barrier. The wider implications of the prospect of cell based treatments are summarized in a review article where the coining of the effort and/or subject of Regenerative Medicine is presented (Daar et al. 2007).

The CNS has attracted attention since the potential of restored function could be very valuable for patients. Particularly since pathological conditions in the CNS can be severely disabling. Cell based therapies are in clinical trials in *e.g.* Parkinsons Disease (Freed et al. 2001; Gordon et al. 2004), ischemic stroke (Kondziolka et al. 2000; Bang et al. 2005) and spinal cord lesions (Sykova et al. 2006). Outside the CNS, other clinical trials with cell based therapies aimed at *e.g.* muscle dystrophy (Gussoni et al. 1997; Miller et al. 1997), ischemic heart disease (Stamm et al. 2003), graft versus host disease (Le Blanc et al. 2004; Ringden et al. 2006) and type I diabetes mellitus (Scharp et al. 1991; Shapiro et al. 2000; Korsgren et al. 2008) have yielded promising results. So far, many of the cell therapies are still in trials since both safety and effects must be thoroughly evaluated.

In many areas of pre-clinical, and to some extent clinical research, cell based therapies deliver positive results. However, as with the definition of stem cells/progenitor cells (Potten et al. 1990) the field of cell based therapies are a very heterogeneous one (Bajada et al. 2008). One feasible way of applying taxonomy to this field is by discussing different basic components in the treated diseases. The first example would be in pathological conditions with a definable population of cells being defect. Examples of diseases with certain cell types being depleted are Morbus Parkinson - dopamine producing cells, (Lindvall et al.), muscle dystrophy -satellite cells of the muscles (Gussoni et al. 1997) and type I diabetes - insulin producing cells (White et al. 2001). Such specialized cells might be easier to replace than the second general idea of cell transplantation wherein attempts of transplantation is aimed at more intricately functioning physiological systems. The complexity increases steeply when transplanted cells must differentiate into subpopulations of cell and/or interact within networks (*e.g.* the CNS). An example of that would be the transplantation of neural progenitor cells with the aim of full neural integration (Nikolic et al. 2009). A third strategy is to exert effects on existing cells/organs through transplantation but without functional integration. This strategy might explain some of the results from studies aimed at integration of cells, but without engraftment in the target organ, albeit with positive functional results observed (Borlongan et al. 2004). One explanation for that phenomenon is that in more complex situations, such as following CNS insults, some of the reported beneficial effects might be associated with immune modulation or local secretion of growth factors, thereby rescuing cells from apoptosis. Examples of immune modulation could be Fas-ligand expressing cells (Ghio et al. 1999; Nagata 1999; Lee et al. 2008) whereas secretion of growth factors could be exemplified by an over-expression of IGF-1 from transplanted mesenchymal cells (Haider et al. 2008). Modulation of the immunological response by cell transplantation has also been shown to favorably treat graft versus host reactions in clinical practice (Le Blanc et al. 2004; Ringden et al. 2006). The concept of transplantation of cells serving as self renewing, local, biologically active, pharmacological factories are attractive for many parts of regenerative medicine (Amar et al. 2003). Local, self-sustaining, treatments that are only affecting niche parts of organs have many benefits that might include, but are not limited to, higher local concentration, less risk of adverse events and customization to different pathological conditions.

1.2 Delivery of cells

For cell transplantation, different percutaneous techniques assisted by modern imaging are viable through minimal invasive methods (Bale et al. 2007) and most parts and locales of the human body can be reached with that approach. On the other hand, for organs with less accessible anatomical location, parenchymal access can be associated with significant surgical risks (Villiger et al. 2005; Ben-Haim et al. 2009). In situations where engraftment rate after intravenous or intra-arterial cell administration is low and when a high anatomical specificity is required, such as the scenario when replacing a distinct cell type, direct puncture of the parenchyma might be preferable. For CNS applications this can be done with stereotactic needle puncture or in a combination with open surgery (Hagell et al. 2002; Wennersten et al. 2004).

Direct parenchymal access can also be achieved by endovascular technique. An example of this is a system that adds a possibility to, via large veins, administrate cells to the heart parenchyma (Thompson et al. 2003). The design of that system, requiring a large diameter catheter and without a closure device for the penetration site makes it usable only in large vessels on the venous side, more specifically, in the coronary sinus of the heart (Thompson et al. 2003; Siminiak et al. 2005). Other organs that might be difficult to reach, such as the CNS and the pancreas, are not reachable by the transvenous technique due to the design of that system requiring a large catheter diameter. Furthermore, venous navigation to most parts of the CNS and the pancreas, and to certain parts of the heart, is very difficult due to the more unpredictable venous anatomy and the venous valves.

Insulin producing cells are today transplanted by a hybrid method with percutaneous access to the portal vein and then intra-luminal cell release in the bloodstream. The concept of the intra portal transplantation is considered superior to open surgical techniques, due to the un-acceptable risk of adverse events (Kandaswamy et al. 1999; Humar et al. 2000). The risk-analysis naturally differs substantially for different surgical procedures and transplantations, both with respect to organs and cells. Risks with portal vein transplantation include portal vein thrombosis, hemorrhages, and transient increase of transaminase values (Shapiro et al. 1995; Ryan et al. 2001). A continuous work of reaching a balance between the risk of bleeding after the procedure and portal vein thrombosis is of utmost importance for portal vein transplantations and results are improving steadily in clinical trials. Further adding to the risk side of the comparison is the need for immunosuppressant treatment after transplantation. The potential benefit of the procedure must also, as in the case with diabetes and insulin producing cells, be compared to the standard treatment of insulin injection and or pumps. Patients eligible for portal vein transplantation of insulin producing cells are thus: patients already subject to immunosuppressive therapy due to previous transplantations, patients with unstable glycemia, unawareness hypoglycemia, or patients with progressive chronic complications despite intensive insulin treatment (Bertuzzi et al. 2006). It has been suggested that it would be of great benefit if insulin producing cells could be transplanted directly to the parenchyma of the pancreas. Advantages with pancreas as the target locale are *e.g.* the possibility of mimicking the physiological release of insulin and the more hospital micro-environment for the insulin producing cells; the pancreas has a higher oxygen tension compared to the liver (Merani et al. 2008).

Different strategies of cell based therapies are currently being evaluated in both pre-clinical and clinical trials but the cell delivery methods per se have received limited interest. One of

the larger obstacles that have been observed, is that the lung acts as a kind of clearance filter for the intravenous cell infusion, resulting in pulmonary trapping (Barbash et al. 2003; Fischer et al. 2009). An intra-arterial selective approach would possibly result in higher transplantation efficiency in certain conditions. The versatility of cell suspensions must not be underestimated and limit the way of thinking when considering treatments with cell based approaches (Nikolic et al. 2009). The cell suspensions can easily be handled and administrated through tubing and catheters, thus providing the possibility to by-pass the lung and selectively reach designated target vessels/parenchyma with a first passage effect. Those possibilities of cell handling forms the basis for catheter based strategies for cell transplantation.

Endovascular treatments are continuously providing a third option to open surgical or percutaneous approaches. From the establishment of the Seldinger technique (Seldinger 1953) and the first use of digital subtraction angiography (DSA) (Meaney et al. 1980) to the modern interventional lab with 3D road maps (Soderman et al. 2005) and CT like capacities of the C-arm (Soderman et al. 2008) the path has been long but rapidly progressing. The driving force up until today, that has made the leap ward style of improvements possible, is both rapid developments in computational power and material sciences. The arteries and veins can today be regarded as “internal routes” for navigation, diagnosis and intervention. The shift from open surgical options is for example illustrated by the patients that used to undergo thoracotomy and that now are being referred for percutaneous coronary intervention or the established coiling of intracranial aneurysms instead of open neurosurgical operation.

1.3 Scaling from bench to bedside

As this chapter hopes to illustrate; the rapid development of endovascular technique has implications on cell transplantation methods as well. To illustrate these implications as opposed to organ transplantation, one can visualize a liver transplantation. The wound in the abdominal wall must at least be big enough for the liver to go into the patient. This severely limits the possibility of minimal invasive transplantation of organs. On the other hand, the versatility of cell suspensions could make intra-luminal techniques the natural way of access. In experimental trials, open surgical options are used in e.g. rodent models for transplantation with positive results. This presents a limitation of scalability for clinical translation. For instance, when evaluating pre clinical CNS transplantation schemes in rodents one or two burr holes are established and cells are transplanted. One or two injections in the rat brain covers a relatively large volume but when scaling that to the human brain following e.g. a middle cerebral artery ischemic event, a large number of percutaneous trajectories would be required to cover a human brain volume corresponding to the experimental situation. The migratory capacity for transplanted mesenchymal cells after stereo-tactical transplantation has been shown to be around two millimeter over 14 days (Chen et al. 2001). Furthermore, the mechanical neuronal injury and the risk for intracerebral hemorrhage would increase with each injection trajectory. In brain stimulation procedures, the literature is somewhat divergent; the risk for hemorrhage could be as high as 5% per injection (Ben-Haim et al. 2009). The easier clinical scalability of endovascular access comes in the terms of cells delivered to a larger volume of tissue by taking advantage of the already existing vascular system. If an ischemic stroke

occurs due to a vessel occlusion at some point, it would be very tempting to intraluminally disperse cells from the same point via the vascular system in order to reach the affected parenchyma. A normal cell dose for a human adult would probably at least be hundredfold higher than in the rodent and needs to spread out over a vastly larger volume thus requiring many injection trajectories. The average human brain weight is quoted at around 1400 grams as opposed to the adult rat at 2 grams to give some sense to the scale proportions.

Endovascular intervention is not without risks either, exemplified by the risk of adverse events reported at 0 to 4.0%, commonly reported at 0.5%, in different cerebral interventions (Raymond et al. 1997; Cognard et al. 1998; Ng et al. 2002; Murayama et al. 2003; Gonzalez et al. 2004; Cronqvist et al. 2005). Added to the risk of the procedure *per se* is the risk of cell transplantations. The risk of intra-arterial transplantation has already been documented in humans for up to 24 months without severe complications, albeit in a small material (Sykova et al. 2006). Other pre-clinical studies with intra-arterial coronary injections performed in healthy dogs revealed micro-infarction of the heart parenchyma (Vulliet et al. 2004) whereas that has not been observed in clinical studies (Stamm et al. 2003). Factors that might be limiting are the size of the cells injected versus the size of the capillary system, the proportion of shunts in the microcirculation, the stickyness of the cells and the amount of cells administered. Many of the cells have a much larger diameter (10 up to 70 μm) as opposed to the capillaries 5 to 8 μm (Chien et al. 1975). The shunting zones in the microcirculation could potentially lead also large cells to the post-capillary venules where diapedesis usually occurs (Tuma 2008). The main reason for leukocyte adhesion/diapedesis through venules is the usually restricted expression of adhesion molecules on venular but not arteriolar or capillary endothelium (Tuma 2008). In intravital microscopy studies, adipose mesenchymal cells have been shown to act as embolic material (Furlani et al. 2009). This risk could be speculated on to be lower than the comparable trauma of the surgical methods although a final risk assessment requires a randomized clinical trial.

1.4 Leaving the bloodstream - diapedesis

One limiting factor to specific intra-arterial and intravenous transplantations is that an intra-parenchymal approach yields a higher efficacy. It has been shown that when performing transplantations to a rodent stroke model and comparing intravenous, intra-ventricular and intra-striatal injections, the highest efficacy in sheer number of cells were obtained with the intra-striatal route (Jin et al. 2005). Further limiting the selective intra-luminal approach is the speculation that some cell systems, such as insulin producing cells, appear incapable of leaving the bloodstream (Hirshberg et al. 2002).

In all implementations aimed at intra-luminal administration (intra-luminal encompassing both intravenous and selective intra-arterial administration) the ability of the cells to leave the bloodstream, or perform diapedesis (Fulton et al. 1957) is fundamental. The diapedesis function has previously been studied predominantly in immunological cells (Fulton 1957), it is in fact the active process whereby cells leave the bloodstream. The diapedesis of immunologically active cells has been thoroughly studied since the discovery of the significant multistep, ordered, cross-talk procedure of leukocyte-endothelial cell interaction both *in vitro* and *in vivo* (Butcher 1991; Springer 1994).

The barricades limiting the cells from haphazardly leaving the bloodstream are many. All blood vessels contain an endothelium and several organized barriers. In the CNS for instance, tight junctions are located between endothelial cells, predominantly to permit the conservation of the water fraction of the blood stream. The liquid pressure gradient composed of the blood pressure can be broken down to one force directed with the axis of the laminar flow of blood and one force aimed perpendicular to the flow on to the wall (Glagov et al. 1992; Fay 1994), thereby providing an evolutionary rationale for sealing the blood stream tightly. Situated underneath the endothelial cells and forming their structural base is the basal layer; a specific protein structure composed of extra cellular matrix (ECM) proteins abundant with expression of elastin, laminin and collagen type I, III and IV (Mayne 1986). Collagen and elastin are the major structural components of blood vessels of all sizes throughout the mammalian body. The cross-banded fibrils in the tunica media and tunica adventitia, formed by type I and type III collagen, provide the tensile strength and comprise probably 80 to 90% of the total collagen present. The other major structural protein component in elastic arteries is elastin; the protein that provides the elastic component of the blood vessels (Mayne 1986). Around most capillaries, pericytes are situated which further seal the blood stream. The pericytes are less abundant in the post capillary venules where most of the diapedesis occurs. As a general note, the post capillary venules are the most “leaky” part of the vascular tree. It should be noted that blood vessels are not merely the plumbing in the mammal body, they are in all respects a vividly living part of the organism, reorganized as a response to stress and demand (Gibbons et al. 1994) and among other things have a self-generating electro potential towards the bloodstream that is important for the clotting cascade (Danon et al. 1976).

As previously mentioned, in a diverging literature, it has been speculated that some cells totally lack the function of exiting the blood stream, thereby limiting intra-luminal based techniques. Even though insulin producing cells appears incapable of leaving the bloodstream, they are still transplanted through the portal vein (Hirshberg et al. 2002). The mechanism of action is believed to be microembolization of the cells to the liver parenchyma in the low pressure system that the portal vein constitutes (Lehmann et al. 2007). It has been speculated that arterial blood from the hepatic artery pushes back into the portal vein during the mixing of the two flows which would stop the cells and create advantageous conditions for engraftment. The cells found functioning in the livers of transplanted patients are, however, situated as plaques in the hepatic artery tree thus adding yet another hurdle to the understanding of portal vein transplantation. The hypothesis is that the cells cannot perform diapedesis but are instead primarily forming a mural thrombus after, with low probability, being displaced against the blood flow into the arterial tree and then encapsulated by endothelial cells. The plaque formation can thereby provide capillary ingrowth, an absolute requirement for endocrine function (Korsgren et al. 2008). Such a hypothesis would also shed further light on the low efficacy of portal transplantations methods. Nevertheless, the portal vein approach to transplantation is today the golden standard since the existing open surgical options of total pancreatic transplantation carries a mortality risk of 10% during the first year of follow-up (Kandaswamy et al. 1999; Humar et al. 2000).

The process of diapedesis is basically divided into tethering, rolling and stopping of leukocytes prior to diapedesis into inflamed tissue. It is thought of as a multistep procedure

involving complex crosstalk between cells in the bloodstream and the endothelial cells. On the endothelial side intra-cellular adhesion molecule -1 (ICAM-1) (Dustin et al. 1986; Rothlein et al. 1986), vascular cell adhesion molecule -1 (VCAM-1) (Elices et al. 1990; Pulido et al. 1991) and junctional adhesion molecule -A (JAM-A) have all been implicated to play crucial roles. Interestingly, all of these receptors are part of the Ig-super family. Leukocytes capacity for homing and diapedesis is pivotal for the development of the inflammatory response to injury and starts as a tightly controlled up-regulation of endothelial E- and P-selectins that stimulate the leukocytes (Vestweber et al. 1999). These leukocytes then respond by activation of G protein-coupled receptors that increase the affinity for endothelial VCAM-1 and ICAM-1 (Muller 2003). Interaction with VCAM-1 is maintained through the heterodimer CD29 and CD49d forming the cell surface antigen Very Late Antigen - 4 (VLA-4) (Elices et al. 1990) and the CD11aCD18 heterodimer interacting with ICAM-1 (Meerschaert et al. 1995). VCAM-1 and ICAM-1 have previously been shown to be up-regulated on the endothelium as a response to inflammation (Dustin et al. 1986; Pulido et al. 1991). This interaction starts the diapedesis itself wherein the leukocytes “crawl” through, either a para-cellular or a trans-cellular pathway interacting with both PECAM-1 and/or CD99 or members of the JAM family of proteins (Petri et al. 2006). Thus significant crosstalk is needed to initiate and execute the active process of diapedesis.

VCAM-1 is also implicated both as a model of treatment for immunological modulation of the inflammatory responses following CNS insults and as surface antigen for cell treatment of CNS insults. In one study, fluorescence activated cell sorting (FACS) was performed for the expression of CD49d and identifying it as one of the important factors for directing diapedesis (Guzman et al. 2008). This finding has received a lot of interest from different fields, among other it has been shown that VCAM-1 expression has a critical role in transplantation of cells in dystrophic muscle (Gavina et al. 2006). Another utilization of VCAM-1 is the selective blocking by the monoclonal antibody drug Natalizumab used in multiple sclerosis, thereby inhibiting diapedesis for immunologically active cells (Stuve et al. 2008). VCAM-1 blockade has also been tested for neuroprotective effects following ischemic events in pre-clinical trials with disappointing results (Justicia et al. 2006).

2. Results and discussion with emphasis on the central nervous system

In this chapter three different types of endovascular methods of cell transplantation are discussed. The first two are the selective intra-arterial and the intravenous methods, these are compared for efficacy. The third method is the trans-vessel wall technique by using the Extroduder; an endovascular catheter system developed within our group for penetrating the vessel wall from the inside to out, thereby creating a working channel to extravascular tissue.

For certain cell types, the selective intra-arterial method is superior to the intravenous one after TBI in the rat. This was measured by the level of engraftment of hMSC at one and five days following TBI, without thrombo-embolic complications (Lundberg et al. 2009). Selective intra-arterial transplantation method for rNPC after TBI in the rat is also superior compared to intravenous method. We were, however, not able to engraft hNPC, *ceteris*

paribus, thus indicating that diapiesis and engraftment is an active process and that different cell systems have different capabilities to engraft following intraluminal delivery (Lundberg et al. 2011). We show, by indirect methods, that CD29CD49dVCAM-1 interactions might be one of the factors with impact on engraftment in an intra-luminal transplantation setting (Lundberg et al. 2011). Further, we show that it is possible to perform minimally invasive parenchymal injections by trans-vessel wall technique by the development of the Extroducer (Lundberg et al. 2010). The Extroducer have no adverse long term effects on the blood vessels up to 3 months following interventions (Lundberg et al. 2011) and it is feasible to use the system as a novel approach for transplantation of e.g. insulin producing cells to the CNS, to the pancreas or other cell types to organs that are difficult or risky to reach by traditional methods. The trans-vessel wall technique thereby adds a new possibility when transplanting cell populations without the necessary properties for performing diapiesis. The establishment of a working channel to the extravascular tissue, by endovascular method, also opens up several other possible applications, such as different methods for sampling.

2.1 Selective intra-arterial method versus intravenous method

We established a model for selective intra-arterial transplantation (Lundberg et al. 2009) that we applied to a model of TBI in the rat (Feeney et al. 1981). We compared the selective intra-arterial method to intra-venous methods for different cell systems in the same TBI setting. Several different variables were tested such as cell concentration, days post injury for transplantation, time for infusion, level of immunosuppressant drugs etc. The majority of transplantation experiments were not successful from the start and many different variables were tested before robust engraftments could be reached. Too few cells had a dire impact on the success of transplantation; 200.000 (unpublished results) and 500.000 hMSCs did only result in very low engraftment. The failure of low cell numbers can obviously be interpreted as an indication that the efficacy of the intra arterial method in this setting is quite low although it is still superior to intravenous alternatives.

Results of engraftment levels were obtained through IHC methods by counting engrafted cells in sectioned brains (Fig 1). We found that engraftment levels of rNPCs were more than five-fold higher than in the control group ($p=0.034$) and hMSC were more than fifteen-fold higher than the in control group in absolute values ($p=0.007$), with a large spread within the intra-arterial groups (Fig 2). Few studies compare selective intra-arterial and intravenous methods but recent clinical data suggest an advantage for the selective intra-arterial route (Sykova et al. 2006). A known problem with all intravenous methods is the fact that the lung acts as a kind of clearance filter during the first passage (Barbash et al. 2003; Fischer et al. 2009). Following intravenous cell infusion, the blood transports the cells after venous passage to the right ventricle and then through the lung where up to 80% of cells are trapped during the first passage (Fischer et al. 2009). Thus as low as 20% of the cells transplanted might be ejected for the first total body distribution through the aorta. Of all the blood leaving the left ventricle of the heart, only somewhere between 1.8 to 8.5 percent of the blood actually reaches the brain of the rat (Pannier et al. 1973) leaving only 0.01 to 1.7 percent of the initially transplanted cells to reach the brain in the first passage. All cells not distributed to the brain are then again re-transported e.g. by PET for mesenchymal stem cells acting as a filter. This phenomenon has been studied e.g. by PET for mesenchymal stem cells

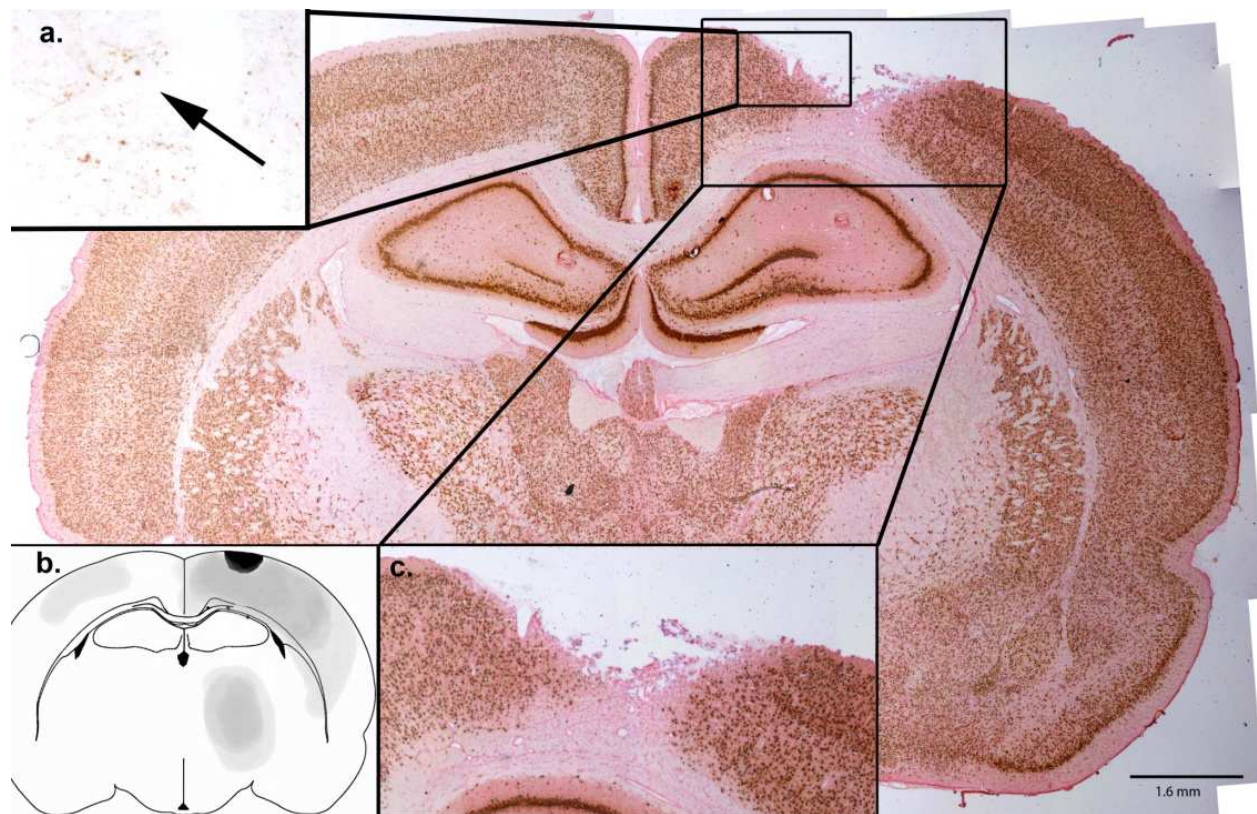


Fig. 1. Representation of intra-arterial transplantation in the TBI model

In the background is a reconstructed image showing a coronal section of the rat brain 2.5 mm posterior to bregma stained with GFAP (red) and NeuN (Brown) five days after traumatic brain injury. In blow-up a. a low magnification single staining with HuN (human nuclear antigen, MAB 1281) without any counterstaining, along the peri-lesional zone is shown. Brown dots represent HuN positive, transplanted cells (arrow indicates an example of a positive cell). In blow-up b. the black area represents the contusion zone and the grey areas represent primary localization of engrafted mesenchymal stem cells. In blow-up c. a magnification of the injury area itself is presented.

(Ma et al. 2005). This situation can readily be changed by placing a micro-catheter in the arteries supplying the organ of interest. An interesting finding in the present study is that our hMSCs predominantly were found in the spleen with few transplanted cells in the lung at 24 hours post injection, a bio-distribution phenomenon, from the lung to the spleen, previously described in rat following intravenous hMSC transplantation (Detante et al. 2009). To increase the understanding of the role of cell line properties for engraftment, we conducted a study with transplantation of different cell lines through either intra-arterial or intravenous routes. This analysis showed that there were dramatic differences between the different cell lines; hNPCs did not engraft at all after intra-luminal delivery whereas there was a significant difference between the hMSCs and the rNPCs using the same transplantation method. Noteworthy is the large variability in the engraftment levels of the latter two cell lines. One important factor that might contribute to this is that neither hMSC nor rNPCs are defined homologous cell lines, succinctly there can be important variations within the cell systems transplanted. The remarkable finding that no engraftment was obtained following hNPC transplantations is even more noteworthy since the hNPCs have

previously been robustly transplanted by open surgical technique (Wennersten et al. 2004; Akesson et al. 2007).

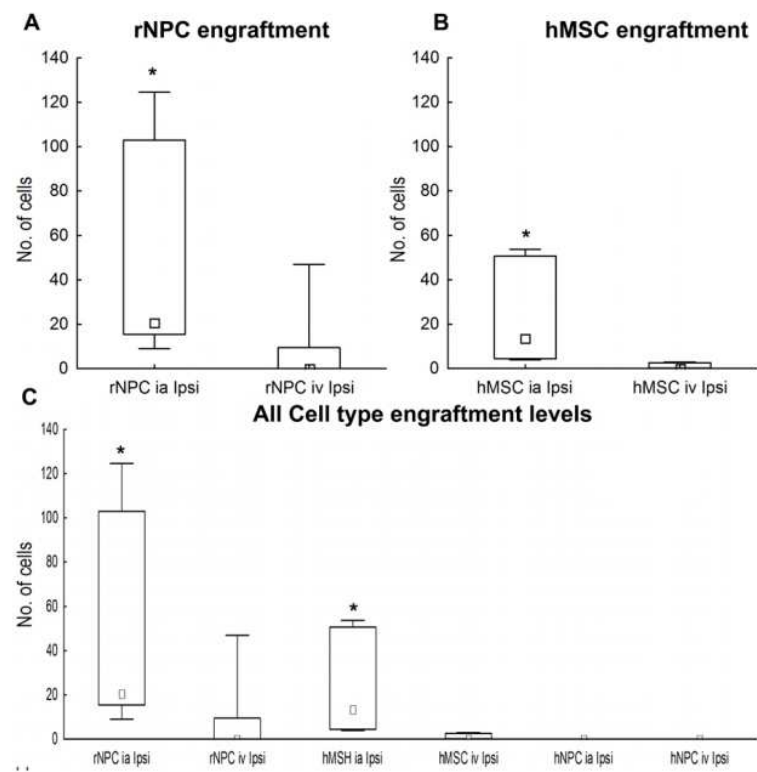


Fig. 2. Graph illustrating engraftment levels
Engrafted cells were counted per section and is reported with median (marker), quartiles (box) and max – min (whiskers). In A a significant difference between engraftment levels per section following selective intra-arterial and intravenous transplantation in the ipsilateral hemisphere of the rNPC group. In B a significant difference between engraftment levels per section following selective intra-arterial and intravenous transplantation in the ipsilateral hemisphere of the hMSC group and finally in C a panel of all engraftment levels per section in the ipsilateral hemispheres of all groups. * marks $p < 0.05$

2.2 Gene expression profiling of cell systems

After discovering that there were differences in engraftment capability between the different cell lines, the opportunity to investigate the bio-molecular basis of said differences presented itself. We started with characterizing and confirming earlier results (Clausen et al. 2007) that our TBI model leads to up-regulation of VCAM-1 expression in the endothelium. That result may suggest that the injured CNS parenchyma could provide cues for diapedesis and migration of engrafted cells in similar ways as immunological cells respond to inflammatory cues (Butcher 1991; Springer 1994). As a screening method, we started by performing microarray on the human cells. rNPCs were not included in the microarray analysis due to problems with cross-species comparisons in the microarray chips used. hMSCs showed a broad expression of integrins, commonly expressed by immune cells, that are important for diapedesis through the vessel endothelium and subsequent migration into the parenchyma. Specifically, analysis of the heterodimers forming receptors for ICAM-1 and VCAM-1 were

analyzed, based on previous work indicating CD49d expression as important for successful intra-vasal transplantations (Guzman et al. 2008). Thus, probably the most interesting finding was the CD49d signal of 68 in hMSC as opposed to 0.4 in hNPC ($p=0.0047$). This was then confirmed with RT-qPCR data from all cell lines with average CD49d mRNA levels that were highest in the hMSC (0.98) followed by the rNPC (0.0057) and finally a dwindling finding in hNPC (0.0012). CD29 and CD49d forms a heterodimer named very late antigen -4 (VLA-4) which was expressed in falling order in hMSC, rNPC and hNPC (Fig 3). The difference in mRNA levels between rNPC and hNPC is not large but might reflect larger differences in protein translation. Further studies at the protein and functional level are required to elucidate the importance of CD49d for diapedesis of these cell systems. CD11a mRNA was detected in hNPC (0.0029) albeit with low CD18 mRNA (0.00025), suggesting that CD11a-CD18-ICAM-1 interaction may be dispensable for engraftment. In contrast, hMSC displayed high CD18 mRNA levels (0.43) but no detectable CD11a expression, suggesting neglectable ICAM-1 dependent engraftment in the hMSCs.

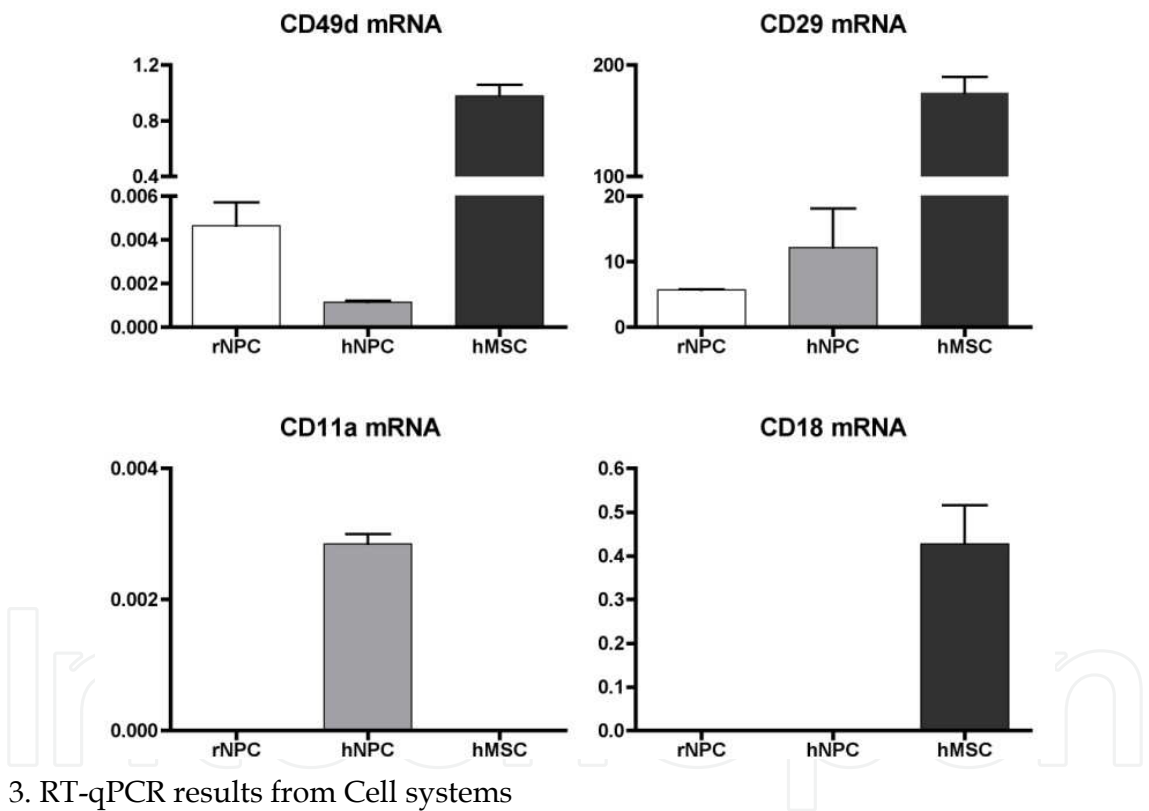


Fig. 3. RT-qPCR results from Cell systems
Bars represent relative levels of integrin CD49d, CD29, CD11a and CD18 mRNA expression in rNPC, hNPC and hMSC compared to respective endogenous TBP mRNA levels. Error bars represent the distribution between the biological replicates.

Finally, both our own findings regarding hNPCs and other previously known cells incapable of diapedesis, such as insulin producing cells (Hirshberg et al. 2002), shows the apparent need for surgical techniques. In some organs that are hard to reach and/or when surgical technique comes with a high risk of adverse events for the patients, the need for an alternative strategy becomes apparent. Thus, we also initiated the development of the trans-vessel wall approach.

2.3 Trans-vessel wall transplantation

An endovascularly based system that could penetrate the vessel wall would, in instances where the target parenchyma is either hard to reach or carries a significant surgical risk, be a method with both the merits of accurate placement and reduction of patient risk. Further, it would solve the problem for certain cells to leave the bloodstream. A proposed solution to this problem is a endovascular catheter system that we have named the Extroducer (Lundberg et al. 2010).

2.3.1 Extroducer in vivo testing - small animals

After extensive computer simulations and *ex vivo* testing, *in vivo* short term testing were performed in rat by creating arterial access from the medial tail artery and performing the Extroducer trans-vessel wall technique passage in either the subclavian or carotid artery. Two different stages of the procedure was tested; first the trans-vessel wall technique passage per se with surgical microscope monitoring of hemorrhage or other adverse events, and thereafter the deployment of the distal penetrating tip through the vascular wall and retracting the proximal part of the system. No cases of intra-operative hemorrhage or intraluminal thrombosis occurred. Thus, the vascular penetration procedure was uneventful and the vessel wall completely sealed around the Extroducer, thereby preventing leakage of blood.

The second group with deposited Extroducer tips also showed absolute hemostasis during the primary intervention. Fourteen days post intervention, this group showed no signs of pain or discomfort. No signs of dissection of the vessels or impairment of blood-flow distal to intervention sites were observed and macroscopical analysis of the organ supplied by the vessel, showed no infarcts.

With computer-based flow simulations, we found that there should be no blood-flow through the detached Extroducer interior lumen at physiological blood pressure. This was also tested *in vivo* by cannulating the deployed distal tip of the prototypes with a nitinol mandrel. This was done to reassure that even when removing possible clotting inside the prototype, it still prevented bleeding from inside the vessel to the extravascular space. Furthermore, no signs of delayed hemorrhage were detected.

2.3.2 Extroducer in vivo testing - large animals

After successful trials in small animals, the Extroducer system was tested in large animals. An adaptation towards clinical use was that these prototypes were manufactured from a longer nitinol tube, 1700 mm *vis-à-vis* 300 mm that was used in the rat. We evaluated the prototypes in the rabbit together with standard clinical catheters and angiographical equipment.

The Extroducer prototypes within the microcatheters were visible at high magnification fluoroscopy and thereby maneuvered into the subclavian artery (SCA) (Fig 4). The rabbit SCA was chosen since it is close to the intended target vessel size of 0.5 to 3 millimeters, the SCA is fairly easy to access in order to perform simultaneous open surgical monitoring and it had been used in the rat. A slight amount of pressure was required on the protecting plastic catheter to advance the system through the microcatheter to the desired vessel wall, thereafter the Extroducer was gently advanced out through the vessel wall to the extravascular space. Hemorrhages were neither observed by simultaneous direct observation

through a surgical microscope, nor by high resolution angiographical series (DSA), during and after the intervention (Fig 4). Further, no thromboembolic complications or vascular dissections were observed using high resolution DSA. No navigational problems were encountered with respect to Extroducer prototype integration with clinical catheters.

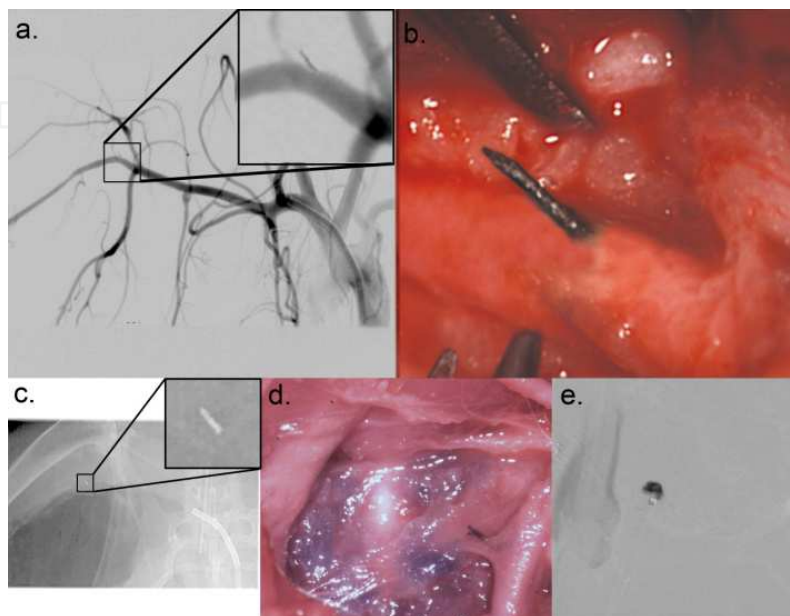


Fig. 4. Trans-vessel wall interventions

For full control over the procedure in the large animal trials, both a surgical microscope and high resolution angiographical series was used. In a. digital subtraction angiogram showing a detached Extroducer tip without hemorrhage, dissection or thromboembolic complications. In b. photograph showing the microsurgical view of the detached Extroducer tip. In c. x-ray image showing the detached Extroducer tip with guide catheter. In d. photograph from post-operative dissection showing the detached Extroducer tip with methylene blue injected in the surrounding tissue. In e. digital subtraction angiogram showing an extra vascular injection of 25 µl contrast agent through the Extroducer system.

Finally, electrolysis detachment of the distal Extroducer distal tip was tested in rabbit. We chose electrolysis since it was the easiest way of performing detachment in our hands. Our design was based on the work of the first detachable coils (Guglielmi et al. 1991). An important difference compared to the detachment zone in coils was, however, that we needed a hollow detachment zone which required additional development. After navigation to the designated intervention site and after methylene blue or contrast agent had been deposited in the extravascular space, a tension of 8V was applied and the distal tip was then detached after, on average, five minutes (range three to nine minutes). This was also uneventful without observation of hemorrhage from around the body of the distal tip or through the inner lumen. The procedure was successfully performed both with simultaneous microscopical monitoring via surgical access, and with fluoroscopical/angiographical guidance solely. In a previous work describing a method for penetrating large veins (Thompson et al. 2003), that system design required a much larger catheter and also lacked a method for sealing the vessel wall, thus making penetration through the arteries impossible. This severely limits the use of that system to their testing

vessel, i.e. the sinus coronarius of the heart, whereas the Extroducer is applicable in both arteries and veins of any sizes down to approximately 0.5 mm in diameter. Another system, in which vessel perforations are performed, is the trans-jugular intrahepatic portacaval stent shunt (TIPS) technique (Richter et al. 1990). That system also does not have the requirement of sealing the vessel wall when finishing the procedure, since a patent blood flow through the stent is the preferred result. On the contrary, thrombosis of the stent might be considered the main problem (Merli et al. 1998) which requires rigorous follow-up.

Thus, the Extroducer system is unique in the ways that it permits safe exit of both arteries and veins and that it is usable in vessels with large dimensions as well as in the microvasculature with inner lumen diameters down to approximately 0.5 mm.

2.2.3 Extroducer testing with long time follow up

In a long term follow up, the end points five days, one month and three months after the deployment of the device was selected. No stenosis or late hemorrhagic complications were noted in any animals. No alterations in behavior or other measures of discomfort were noted either (Lundberg et al. 2011).

The distribution of followed up animals were as follows; two at the five days end point, five at the 30 days end point and six at the 80 days end point with histological analysis of one resulting in a total number of 19 detached Extroducer tips.

In the follow up DSA we also found that four of 19 (21 %) of the detached tips were no longer placed through the vessel wall but had instead been “pushed” or “migrated” through the endothelium to the extravascular space immediately adjacent to the penetration site. No vascular stenosis or other adverse reactions were observed around those tips. Also for the rest of the tips, that were located through the vessel wall, no adverse reactions were detected. An important endpoint was biocompatibility of the detached tips. The nitinol alloy used was selected for its many advantageous properties. Nitinol is a nickel/titanium alloy with both memory and super elastic properties (Adler et al. 1990). These special properties are the foundation for the use of nitinol in stent fabrication in clinical practice. Thus, the excellent biocompatibility of nitinol (Castleman et al. 1976) has been extensively studied, especially with respect to vessel wall interactions in the use of stents (Stoeckel et al. 2004). However, the compatibility of nitinol when placed through arterial walls has, to our knowledge, not been studied. The parylene, used as coating in our device, is also FDA approved and CE marked when used in pacemaker electrodes. To evaluate interaction between the deposited tips and the endothelium, we performed histological analysis with the prototypes *in situ* by a specialized grind-cutting technique and then consulted an external, independent, evaluator with expertise in the field of titanium implants. This evaluation showed full biocompatibility with a very small fibrotic capsule ($< 1 \mu\text{m}$) formed around the detached distal tips (fig 5). No ongoing inflammation was observable around any of the distal tips. Around one (of the total number 14 left in place) of the day five animal implants, three macrophages were indentified in the area of the detached distal tip. Apart from those three macrophages, no other signs of inflammation were observed. The endothelia showed no signs of alterations adjacent to the deposited tips.

In conclusion, the biocompatibility of the distal tips was comparable to titanium implants. The interactions of nitinol with the interior of the vascular wall and the extra vascular space has not been as extensively studied since stents most often are positioned inside the vascular

lumen with direct contact only with the bloodstream and the endothelium. Therefore, the present histological analysis in this new application and new position of nitinol, adds important knowledge about biocompatibility for possible future applications.

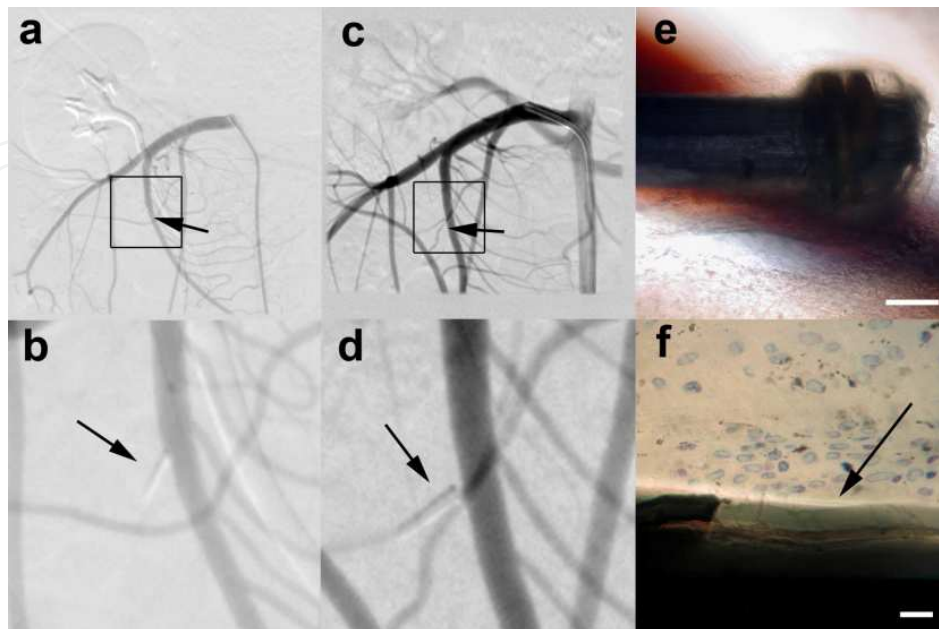


Fig. 5. Long term follow-up of the trans-vessel wall intervention

In a. the initial follow up angiogram directly following detachment in the Superior Mesenteric Artery (SMA) is shown with a square marking the blow-up in b. Arrows indicate the detached distal tip. In c. an SMA angiogram, performed 80 days after the intervention in the same animal, is shown with a square indicating the blow-up in d. Arrows indicate the detached distal tip. In e. a microphotograph of a histological van Geeson and toluidine blue staining prepared by grind-cutting with the detached tip in situ, is shown. Scale bar = 100 μm . The blow-up in f shows the parylene coating surrounding the detached tip which is marked by an arrow. Note that no fibrous response or inflammation is observable. Scale bar = 4 μm .

The prototypes excluded due to failed detachment were analyzed by sweep electron microscopy (SEM), since monitoring of electric current gave limited prognostic information about the failure to detach. We found that, in failed detachments, large amounts of chloride were observed by surface spectroscopy (6-10 wt %) indicating titanium chloride ion formations in a passive layer, thus providing current transmission without electrolysis. Titanium chloride molecules can provide a surface area that lets electron pass through but without formation of soluble titanium or nickel ions. This problem is probably due to the simple technique used for creating the insulation defect in our hands. However, numerous available solutions for detachment are available on the market that can easily be integrated with the trans-vessel wall technique in an industrialized manufacturing process.

3. General discussion

The rationale for our previous studies within the field of endovascular cell transplantation is the path to translating cell based regenerative medicine to patients. In a translational

perspective the actual route of transplantation will be important. For operative techniques there is a problem with scalability and for intravenous techniques there is a problem with efficacy. We show that by selective intra-arterial methods it is possible to increase the level of cerebral engraftment with certain cell types with six to fifteen fold yields. Further, not all cells are optimal for intra-luminal transplantation. It is, for example, known that insulin producing cells lack the capability to perform diapedesis (Hirshberg et al. 2002) and we show that hNPC, a cell system previously transplanted by open surgical means (Wennersten et al. 2004) also lacks the capability to perform diapedesis. For these cell systems, and other applications, we have developed the Extroducer as a tool to establish a direct, minimal invasive working channel with parenchymal access in organs that are difficult or risky to reach with traditional techniques.

The first passage of cells delivered through selective intra-arterial approaches, compared to systemic intravenous delivery, results in a higher local concentration, shorter blood stream exposure and less mechanical stress factors before cells reach the target site. These factors could be of importance for successful engraftment. Supporting that hypothesis are the present results showing significantly higher total cerebral uptake of cells after intra-arterial compared to intra-venous administration. The next supporting fact is the higher uptake of cells in the ipsilateral hemisphere after intra-arterial administration and by the absence of difference between the hemispheres after intravenous transplantation. Future studies are needed for elucidating molecules responsible for diapedesis, e.g by direct methods such as knock-ins, of for example CD49d, in non-functioning cell systems and knock down or blocking in other cell systems.

The absence of adverse effects in transplanted animals suggests that in the short term, the selective intra-arterial transplantation method is safe for delivering even high concentrations of cells. It has been reported ischemic events following intra-arterial approaches to the heart in dogs (Vulliet et al. 2004) thus indicating the need for thorough studies prior to translation into clinical practice. However, in clinical studies on intracoronary infusions (Stamm et al. 2003) and in spinal cord artery infusions (Sykova et al. 2006) no embolic events have been recorded. Connected to the need for safety studies, it could be argued that the higher engraftment rates in our intra-arterial groups would be a consequence of microembolization of cells. In that scenario we would, however, have detected ischemic histological changes and localization of the transplanted cells within arterioles and capillaries, which we did not. For all intra-luminal approaches, this thesis shows that a thorough investigation must be performed to clarify if the cells actually can perform diapedesis prior to choosing transplantation strategy. hNPCs has previously been shown to have an impact on neurological outcome when transplanted with open surgical techniques, but in their present form they seem unsuitable for intra-luminal transplantation. Therefore, based on the findings in this thesis, considerations should be made that CD29CD49dVCAM-1 interaction is one of the best studied crosstalk mechanisms on how immunological cells leave the bloodstream to perform homing to disease ridden tissue where inflammation occurs (Elices et al. 1990). This line of reasoning is supported by other studies implicating VCAM-1 interaction in both ischemic stroke and muscle dystrophy pre-clinical studies (Gavina et al. 2006; Guzman et al. 2008). An interesting comparison can be made regarding diapedesis in cell systems aimed for transplantation compared to immunological cells. In such an exercise it could be considered blatantly ignorant to immediately dismiss the phylogenetically conserved mechanism of the immunological system and suggest a hitherto unknown

method of diapedesis for these cells. Applying Occam's razor to the hypothesis of diapedesis for cell transplantation, two conclusions can potentially be made; i) the process of diapedesis is most probably an active process for cell systems transplanted, since there are no known inactive ways of diapedesis and ii) the most likely system for diapedesis crosstalk should be found within the same systems that are used by the immunological system, meaning that proteins are highly likely to come from the Ig-super family such as VCAM-1. The other, more complex, explanation would be that a hitherto unknown system for diapedesis exists. That it also unlikely since mutations in such a system would lead to genetical diseases that should be known, but without reasonable explanations. A research program dedicated on endovascular transplantation of different cell systems in different diseases should include a variety of diseases and cell systems to increase the understanding of both cell-endothelium interactions and the effects on target niches.

When designing both pre-clinical and clinical cell transplantation studies, consideration should be taken to how the cells are hypothesized to reach their designated targets. The first option might be open surgical techniques for small niche locations in a target parenchyma. Downsides to open surgical/percutaneous techniques are *e.g.* impracticalities of accruing the desired extent of target tissue volume, especially if the volume is relatively large, such as following a major CNS insult. Further, the patient risk for adverse events might be unacceptable in relation to a potential benefit, in particular for "difficult to reach" organs such as the CNS, the pancreas and/or the heart. If the disadvantages of open surgical/percutaneous techniques are unacceptable, intra-luminal options could be explored. For intra-luminal approaches, the concept of how transplanted cells are presumed to leave the blood stream becomes an issue with several potential solutions. For a cell system without the necessary features for diapedesis, one could use knock-in methods to provide the necessary adhesion molecule set up, otherwise intra-parenchymal injections must be considered. For knock-in methods, an intra-arterial approach would probably still have benefits in efficacy over intravenous methods through the first passage effect and by avoiding pulmonary trapping. As previously discussed, an interesting purely academical calculation when performing intravenous cell transplantations could result in such a low cell dose to the brain as 0.01 to 1.7% after intravenous injection, assuming that 80% of cells are trapped in the first passage through the lung (Barbash et al. 2003; Fischer et al. 2009). This can readily be changed by placing a micro-catheter in vessels supplying the target parenchyma with blood thereby providing a chance for all cells to perfuse the target parenchyma.

For more discrete functioning cells, an intra-parenchymal injection might be even more attractive for reasons such as shielding the cells from the exposure to the bloodstream and accurate anatomical placement. In difficult to reach organs, minimally invasive direct parenchymal transplantation could be performed by the trans-vessel wall technique described within this thesis. That technique might, however, not be suitable for treating a large ischemic lesion in humans. On the contrary, in discrete lesions where only a niche cell needs to be replaced, such as in type I diabetes, where the cells do not possess properties for diapedesis, the Extroducer could potentially really show its worth. The next natural step is transplantation of insulin producing cells to swine before planning for clinical trials.

The Extroducer is not limited to cell transplantation. Its main design is to provide a working channel by endovascular technique to the parenchyma in various, otherwise, inaccessible

organs. Through that working channel, other procedures such as local chemotherapy-, irradiation-, growth factor administration, tissue sampling, electrophysiological diagnostics and thermo-therapy becomes possible. Further, combined with optical spectroscopic analysis, it might even be possible to perform infra-light histological analysis of tissue via the Extroducer.

3.1 Conclusions and future research

We have discussed the rationale for using endovascular methods for cell transplantation and described findings from our group and other groups, showing that selective intra-arterial administration is a safe way, with a short follow up time, to increase engraftment levels compared to intravenous delivery. However, not all cell systems are optimal for intraluminal transplantation. These factors might be dependent on integrin expression and endothelium interactions. For cells that lack the capacity to perform diapedesis, and especially for more specific niche cell systems in organ systems that are difficult to reach, we have also developed a system for trans-vessel wall parenchymal access. The Extroducer system has been evaluated both for long term effects and feasibility for pancreas access. *Ergo*, endovascular intervention should provide a number of methods for efficient and safe cell transplantation in current and future clinical practice. The transplantation method must be decided on a disease to disease, cell to cell and patient to patient manner. Future research should investigate the possibilities of providing cells meant for intravasal transplantation with the necessary properties for performing diapedesis.

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This book documents the increased number of stem cell-related research, clinical applications, and views for the future. The book covers a wide range of issues in cell-based therapy and regenerative medicine, and includes clinical and preclinical chapters from the respected authors involved with stem cell studies and research from around the world. It complements and extends the basics of stem cell physiology, hematopoietic stem cells, issues related to clinical problems, tissue typing, cryopreservation, dendritic cells, mesenchymal cells, neuroscience, endovascular cells and other tissues. In addition, tissue engineering that employs novel methods with stem cells is explored. Clearly, the continued use of biomedical engineering will depend heavily on stem cells, and this book is well positioned to provide comprehensive coverage of these developments.

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